Effects of Ultrasound, Irradiation, and Acidic Electrolyzed Water on Germination of Alfalfa and Broccoli Seeds and *Escherichia coli* O157:H7

HYUN JUNG KIM, HAO FENG, MOSBAH M. KUSHAD, AND XUETONG FAN

ABSTRACT: The ability of power ultrasound, acidic electrolyzed water (AEW), and gamma irradiation to inactivate *Escherichia coli* O157:H7 inoculated onto alfalfa and broccoli seeds was examined. The treatment conditions under which the alfalfa and broccoli seeds treated with sterile deionized water (SW), AEW, ultrasound cleaning tank (UST), ultrasound probe (USP), and irradiation (IR) would retain a germination percentage of >85% were first determined for each disinfection hurdle. *E. coli* O157:H7 inactivation tests were then conducted with the experimental conditions determined in the germination tests to find out the maximum inactivation ability of each disinfection hurdle. AEW treatment at 55 °C for 10 min reduced *E. coli* O157:H7 population by 3.4 and 3.3 log CFU/g for the alfalfa and broccoli seeds, respectively. IR at 8 kGy resulted in a 5-log reduction with seed germination of >85% for both seed types, but a reduction in the length and thickness of the sprouts was observed. None of the ultrasound treatments achieved over a 2-log reduction in *E. coli* O157:H7 population without lowering the germination to below 85%. The results of this study demonstrated that AEW and ultrasound, when applied individually or in combination with thermal treatment at 55 °C, were not able to deliver a satisfactory inactivation of *E. coli* O157:H7. A combination of several hurdles must be used to achieve a complete elimination of *E. coli* O157:H7 cells on alfalfa and broccoli seeds.

Keywords: alfalfa seeds, broccoli seeds, electrolyzed water, irradiation, ultrasound

Introduction

Vegetable seed sprouts, such as those grown from alfalfa, broccoli, mung bean, and radish seeds, are commonly consumed raw in salads and sandwiches because of their nutritional values. However, consumption of sprouts has been linked to a number of foodborne disease outbreaks associated with *Salmonella* and *Escherichia coli* O157:H7 infections (NACMCF 1999). Epidemiological evidence and isolation tests have suggested that seeds used for spouting are the source of these pathogens (Breuer and others 2001; Proctor and others 2001; Fett and Cooke 2003). The current recommendation of the US Food and Drug Administration to sprout producers is to use 20000 mg/L of free chlorine from $Ca(OCl)_2$ to treat seeds for up to 15 min to reduce the risk of pathogens (Rajkowski and others 2003). Such a treatment, which is the only chemical treatment approved by the US Environmental Protection Agency for use on sprouting seeds, can reduce but not eliminate the number of pathogenic microbes and hence the potential proliferation of the surviving microbes during sprout growing remains a hazard to consumers. This has been evidenced by detection and growth of *E. coli* O157:H7 on sprouts produced from seeds treated with 20000 mg/L of free chlorine (Lang and others 2000).

To find out an effective means to secure the microbial safety of sprouts, a variety of seed decontamination methods have been investigated over the years (Fett 2006; Fett and others 2006). The concepts tested or under investigation include chemical treatments (single chemical compound, and/or combination of several chemicals) (Taormina and Beuchat 1999; Lang and others 2000; Weissinger and Beuchat 2000; Soylemez and others 2001; Beuchat and Scouten 2002), natural antimicrobials (Fett and Cooke 2003), ozone (Sharma and others 2003; Wade and others 2003), electrolyzed water (Kim and others 2003), ultraviolet light (Sharma and Demirci 2003), irradiation (Thayer and others 2003; Bari and others 2003a), high pressure processing (Wuytack and others 2003; Ariefdjohan and others 2004), and ultrasound (Scouten and Beuchat 2002). It has been recognized that it is difficult to achieve a complete elimination of pathogens without compromising the viability of the seeds using treatments with a single lethal factor (hurdle). Combination of several lethal factors to disinfect seeds has thus gained interest (Scouten and Beuchat 2002). Among the published studies on combination and sequential treatments, Bari and others (2003a) reported more than 5-log reduction in *E. coli* O157:H7 population on alfalfa seeds treated with dry heat (50 °C for 1 h) followed by irradiation with doses of 2 to 3 kGy. Treated seeds exhibited high germination rate (92% to 93%) but sprout length was substantially reduced. Nevertheless, the results from Bari and others (2003a) suggest that, with a proper combination of disinfection hurdles, it is possible to achieve a maximized microbial inactivation while maintaining seed viability.

Previous studies have mainly focused on means to maximize food borne pathogen inactivation. However, there is a lack of detailed studies on the effect of a disinfectant/hurdle on seed germination under different treatment conditions. In this work, efforts were first made to determine treatment conditions for 3 disinfection hurdles, namely power ultrasound, irradiation, and electrolyzed water, leading to a germination percentage of >85%. With the conditions under which the target germination can be achieved, inactivation tests with *E. coli* O157:H7 were conducted to ascertain the maximum inactivation capacity of each hurdle.

MS 20060087 Submitted 2/8/2006, Accepted 4/14/2006. *The authors Kim and Feng are with Dept. of Food Science and Human Nutrition, Univ. of Illinois, Urbana-Champaign, Urbana, IL 61801. Author Kushad is with Dept. of Natural Resources and Environmental Sciences, Univ. of Illinois, Urbana-Champaign, Urbana, IL 61801. Author Fan is with Eastern Regional Research Center, USDA, ARS, Wyndmoor, PA 19038. Direct inquiries to author Feng (E-mail: haofeng@uiuc.edu).*

doi: 10.1111/j.1750-3841.2006.00064.x

The disinfection hurdles used in this study were either physical methods (irradiation and ultrasound) or an environmentally friendly disinfectant (acidic electrolyzed water, AEW) (Park and others 2001). Among the 3 hurdles, the power ultrasound has long been used in many industrial sectors as a good tool for surface cleaning applications (Mason and Lorimer 2002). Gamma irradiation has also been known for years for its good bactericidal ability. The use of irradiation with doses up to 8 kGy has been approved by the US Food and Drug Administration to control microbial pathogens in seed sprouts (US DHHS 2000). AEW has a strong bactericidal effect against foodborne pathogens due to a combined action of free chlorine (<80 mg/L), low pH (approximately 2.3 to 2.7), and high oxidation-reduction potential (ORP) (approximately 1130 to 1160 mV) (Wang and others 2004). With the experimental results reported in this work, the potential and limitation of each hurdle can be determined. The purpose of this study was therefore to examine the maximum inactivation capacity of power ultrasound, gamma irradiation, and AEW on *E. coli* O157:H7 cells inoculated onto alfalfa and broccoli seeds with treatment conditions corresponding to a germination percentage of >85%.

Materials and Methods

Alfalfa and broccoli seeds

Alfalfa and broccoli seeds were obtained from a commercial supplier (Tomahawk Paper Products Corp., Chicago, Ill., U.S.A.) and were stored at 4 °C in a dry environment. The physical characteristics of each seed were determined. For bulk density measurement (total mass per volume), the mass of seeds with a volume of 100 mL was determined by putting the seeds in a graduated cylinder, which was then weighed with 10 replications. Ten seeds were randomly selected and weighed for the estimation of the mass of individual seed kernels, which was also repeated 10 times. The number of seeds in 1 g was counted with 3 replications.

Preparation of inoculum

Five strains of *E. coli* O157:H7 were obtained from the Dept. of Food Science at Purdue Univ. The strains were the following: 13B88, C7929, and G5303 (apple juice isolate), 204P (pork isolate), and EDL933 (human isolate). Each strain (4 mL, 2% v/v) from a frozen stock was grown in 20 mL tryptic soy broth (TSB, Becton Dickinson, Sparks, Md., U.S.A.) at 37 °C for 18 h. Cells of the 5 strains were collected by centrifugation at 4 °C and 7280 × *g* for 10 min and resuspended in 20 mL of sterile 0.l % peptone and 0.85% saline water with the procedure repeated 3 times. An equal volume (20 mL) of the 5 isolates was mixed to obtain an inoculum (100 mL) containing approximately 10^7 to 10^8 CFU/mL of *E. coli* O157:H7.

Inoculation of alfalfa and broccoli seeds

Each seed type (100 g) was added into the inoculum (100 mL) and gently mixed for 1 min. Seeds were separated over a double-layer cheesecloth supported by a wire screen and air-dried overnight at room temperature under a laminar flow hood (Labconco Purifier PCR Enclosure, Kansas City, Mich., U.S.A.). The pathogen population on the seed was determined as described below. Inoculated seeds with approximately 10^5 to 10^6 CFU/g of *E. coli* O157:H7 were packed into plastic Ziploc® storage bags (S.C. Johnson & Sons Inc., Racine, Wis., U.S.A.) and stored in a refrigerator (4 °C) for 7 d before using in decontamination experiments.

Acidic electrolyzed water treatment

AEW was produced from an AEW generator (ROX-20TA-U, Hoshizaki Electric Co., Sakae, Toyoake, Aichi, Japan). Deionized water and 10% NaCl solution were simultaneously introduced into the equipment chamber to make a solution with NaCl concentration of about 0.1% at room temperature. When the unit indicated dispensing electrolyzed water, AEW was collected from the anode outlet and used within 1 h. The pH and ORP were determined with a pH and ORP meter (AR15, accumet® Research, Fisher Scientific Co., Pittsburgh, Pa., U.S.A.). Total chlorine concentrations were measured with an EPA approved chlorine test kit (PCT-DR, LaMotte Co., Chestertown, Md., U.S.A.). AEW was conditioned in a refrigerator at 4 °C and a water bath at 55 °C to get AEW for treatments at 4 °C and 55 °C, respectively.

For germination tests, 1 g of alfalfa or broccoli seeds was treated with 20 mL (1:20 w/v) of AEW (pH 2.57, ORP 1161 mV, and available chlorine 66 mg/L) with continuous agitation using a magnetic stirrer (Fisher Scientific Co., Pittsburg, Pa., U.S.A.) at a medium speed. A sterile deionized water (SW) treatment was used as the control. The treatments were conducted at 4, 23, and 55 °C for 2, 5, 10, 20, 30, and 60 min to determine the effect of temperature and time on germination. In order to control temperature at 4 and 55 °C during a treatment, a Lauda constant temperature bath with a circulator (R-20/2, Brinkmann Instruments Inc., Westbury, N.Y., U.S.A.) was used. The germination rate was determined after decanting AEW or SW as described below.

A germination rate of 85% was regarded as acceptable and was chosen as the criterion for the selection of treatment conditions in the *E. coli* inactivation tests. Based on this criterion, 5 g seeds were treated with 100 mL AEW (1:20 w/v) for 60 min at 23 °C or for 10 min at 55 °C.

Ultrasound treatment

Two types of ultrasound equipment, cleaning tank and probe, were used in this study. The water temperature in an ultrasonic cleaning tank (40 kHz, 4HT-1014–6, Crest Ultrasonics, Trenton, N.J., U.S.A.) was adjusted to 23 or 55 °C. The water in the tank was degassed for 40 min before a treatment. For the germination test, 60 mL of SW was placed in a 250 mL glass beaker that was fixed tightly with metal strings in the ultrasonic bath. The level of SW in the beaker was below the level of the water in the bath. Three grams of seeds were added to the beaker and treated for 1, 2, 5, 10, 20, and 30 min at each temperature. The microbial inactivation tests were conducted with 5 g inoculated seeds emerging in 100 mL of SW at 23 °C for 30 min sonication or at 55 °C for 10 min sonication, which were chosen according to the results of the germination tests.

An ultrasound probe driven by a Vibra-Cell generator (20 kHz, VC750, Sonics & Materials Inc., Newtown, Conn., U.S.A.) was immersed in a 100-mL jacketed beaker with 60 mL of SW for the germination tests or in a 250-mL jacketed beaker with 100 mL of SW for the *E. coli* O157:H7 inactivation tests. The probe was placed at about 3 cm from the bottom of the beaker for all the ultrasound tests. When the temperature of SW in the beaker reached 23 or 55 °C, 3 g of alfalfa or broccoli seeds were added and treated for 0.5, 1, 2, 3, 5, and 10 min. For the microbial tests, 5 g of seeds were treated for 10 min (alfalfa) or 2 min (broccoli) at 23 °C, and 5 min (alfalfa) or 2 min (broccoli) at 55 °C. A constant temperature bath as described in the previous section was used to control the temperature of water during sonication to ± 3 °C of the target values.

Gamma irradiation

Gamma irradiation was performed with a Gammacell 220 Excell Cobalt irradiator (MDS Nordion 447, Ontario, Canada). The irradiator was loaded in October 2002 with 24000 Curie of Cobalt-60. The irradiation chamber was a cylinder with dimensions of 203 mm (8 in) height and 152 mm (6 in) in diameter. Precise dose distribution in

the irradiation chamber was measured with Gafchromic MD-55 (ISP Technologies, Inc., Wayne, N.J., U.S.A.) and Radiachromic FWT-60 film dosimeters (Far West Technology, Inc., Goleta, Calif., U.S.A.), which were calibrated at the Natl. Institute of Standards and Technology, following a standard procedure. The dose range was ±11% for the dose used in this study. The sample temperature during irradiation was monitored and maintained at 23 °C by air circulation.

Two grams of alfalfa or broccoli seeds were placed into a petri dish (50 × 9 mm, Falcon, Becton Dickinson, Franklin Lakes, N.J., U.S.A.) and sealed with Parafilm (American Natl. Can, Chicago, Ill., U.S.A.). Three petri dishes per treatment at each dose were prepared. All petri dishes of seeds were gamma-irradiated at doses of 0 (control), 0.5, 1, 2, 4, 8, and 12 kGy at room temperature. Right after the treatment, the irradiated samples were tested for seed germination percentage as described below. Based on the results of germination tests, 8 kGy was applied for the *E. coli* O157:H7 inactivation test to treat 5 g of inoculated seeds.

Evaluation of seed germination

Alfalfa or broccoli seeds treated as described above were evaluated for their ability to germinate. Approximately 100 seeds subjected to each treatment were placed on 2 pieces of SW saturated filter paper (90 mm dia, Whatman #1) in sterile petri dishes (100 × 15 mm, Fisher Scientific, Hanover, Ill., U.S.A.). The petri dishes were sealed with parafilm to keep the filter paper moist. Seeds were germinated in the dark at 24 °C for 3 d. Three dishes per treatment were prepared. The number of germinated seeds, which were chosen with the radical becoming visibly protruded from the seed coat by at least 2 mm, was counted and the percentage calculated.

Microbiological test

After each treatment, solutions were immediately decanted from treated seeds (5 g). Seeds were transferred to a Stomacher Bag (Whirl-Pak®, Nasco, Fort Atkinson, Wis., U.S.A.) and pummeled with 45-mL sterile 0.1% peptone and 0.85% saline water (PSW) in a stomacher (Lab-Blender 400, Cooke Laboratory Products, Alexandria, Va., U.S.A.) for 1 min. The mixture was serially diluted with sterile PSW and the diluted samples were surface plated (0.1 mL, in 3 replicates) on sorbitol MacConkey agar (Difco, Sparks, Md., U.S.A.) containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) (Oxoid Ltd., Hampshire, UK). Plates were counted as a unit of CFU/g after incubation at 37 °C for 24 h.

Statistical analysis

All experiments were replicated 3 times except those specifically mentioned. The results were analyzed using the Statistical Analysis System (SAS Institute, Raleigh, N.C., U.S.A.) for one-way analysis of variance (ANOVA) to determine differences in germination percentage and population of *E. coli* O157:H7 on alfalfa or broccoli seeds subjected to each treatment. When there was a significant difference among treatments at $\alpha = 0.05$, the Fisher's least significant differences (LSD) test was performed to distinguish the differences.

Results and Discussion

Physical properties

Table 1 shows the physical parameters of alfalfa and broccoli seeds. The alfalfa seeds were typically oval-shaped, whereas the broccoli seeds were more round-shaped. Alfalfa seed kernels were smaller than broccoli seeds as the number of alfalfa seeds per gram was 513 (CV = 1.3%), and that of broccoli seeds amounted to 333 (CV = 4.4 %). The alfalfa seeds had a bulk density of 814.4 kg/m^3, as measured with the graduated cylinder method, while the broccoli seeds were lighter, having a bulk density of 685.2 kg/m^3. The initial population of *E. coli* O157:H7 cells inoculated onto alfalfa and broccoli seeds were 5.03 and 5.21 log CFU/g, respectively.

Table 1 — Physical characteristics of alfalfa and broccoli seeds

Seed	Bulk density (kg/m^3)	Weight/ seed (mg)	Number of seed in 1 g
Alfalfa	813.4 (CV[a] = 0.79%)	1.9 (CV = 6.7%)	513 (CV = 1.3%)
Broccoli	685.2 (CV = 1.2%)	3.1 (CV = 4.4%)	333 (CV = 4.4%)

[a]CV (%) = coefficient of variation.

Germination percentage

The germination percentages of alfalfa and broccoli seeds treated with SW, AEW, and power ultrasound (ultrasound probe [USP] or ultrasound cleaning tank [UST]) are presented in Tables 2 and 3. Generally, increasing treatment time resulted in a decrease in germination percentages except for the water treatments when the water temperatures were at or below 23 °C. For both alfalfa and broccoli seeds, SW and AEW treatments at 4 and 23 °C did not alter their germination ability. At 55 °C, however, a significant decrease in germination rate was observed when treating the seeds for over 10 min. Beuchat and Scouten (2002) reported that heating alfalfa seeds at 55°C for 10 min yielded germination percentage of 74.0% in water and 56.8% to 84.0% in 3 sanitizers [$Ca(OH)_2$, Tweeen, Tsunami] and their combinations. The higher germination rate of alfalfa seeds in this study compared to that of Beuchat and Scouten (2002) may be caused by differences in cultivar of the seeds, among other factors. The alfalfa seeds seemed to be more sensitive to heat than the broccoli seeds, as can be seen by a lower germination percentage when the seeds were treated at 55 °C for over 10 min for SW and SEW treatments. The smaller kernel sizes of the alfalfa seeds may have resulted in a good heat penetration into the kernel and hence caused more heat damage in the seeds. Beuchat and Scouten (2002) noticed that the combined effect of chemicals with heat to kill pathogens also resulted in loss of alfalfa seed viability. In this study (Table 2), however, the germination rate of alfalfa seeds after treatment with AEW + 55 °C for 20 and 30 min was slightly higher compared to the SW treatment at the same temperature. There is no published data in the literature on the germination of broccoli seeds treated with sanitizers.

Alfalfa seeds were more resistant to ultrasound treatments, as shown by the higher germination percentage compared to that of broccoli seeds when treated for over 2 min for USP and over 5 min for UST (at $\alpha = 0.05$). The only exception was treatment with UST at 23 °C where no significant differences for the seeds treated at different sonication times were found. The mode of action for sonication has been attributed to a phenomenon called acoustic cavitation, which is the formation, growth, and implosion of tiny gas bubbles in a liquid subjected to an ultrasound treatment. The implosion of a gas bubble near a solid-liquid interface can generate a liquid jet, which is a high speed in-flow of liquid with a maximum speed of up to 156 m/s (Leighton 1994). The liquid jet would impinge seed surfaces and cause damage. The smaller kernel sizes of alfalfa seeds would allow the seeds to receive less bombardment from the liquid jets, and hence result in less seed damage.

Seed germination for samples treated with UST and USP were different. Theoretically, since USP treatment has an acoustic energy density (AED) of 46 W/mL, much higher than the ultrasound bath (1.8 W/mL), more cavitation activity and hence more seed damage should be expected for USP treated seeds. This was true for broccoli seeds as shown by lower germination rates

Table 2—Germination percentages[a] of alfalfa seeds[b] after treatment with sterile water, acidic electrolyzed water, and ultrasound

	Treatment[c]									
	SW			AEW			UST		USP	
Time (min)	4 °C	23 °C	55 °C	4 °C	23 °C	55 °C	23 °C	55 °C	23 °C	55 °C
0.5									94.6ab	93.9a
1							89.0a	90.7a	97.1a	94.2a
2	87.3a	89.2a	90.2a	88.2a	90.4a	91.0a	88.7a	93.2a	96.0a	97.2a
3									96.5a	96.3a
5	88.2a	89.2a	90.8a	86.3a	87.0b	90.4a	88.8a	91.2a	94.3ab	97.0a
10	86.3a	90.1a	89.6a	86.9a	87.8ab	89.6a	90.2a	80.7b	92.5b	87.6b
20	88.3a	88.3a	57.9b	86.9a	88.4ab	67.2b	87.3a	15.3c		
30	87.6a	87.9a	20.1c	87.7a	88.3ab	28.0c	89.9a	10.8d		
60	85.9a	89.8a	7.8d	85.0a	87.8ab	6.9d				

[a]Within treatment and temperature, values followed by different letters are significantly different ($P < 0.05$).
[b]Control = 85.4 %.
[c]SW = sterile deionized water; AEW = acidic electrolyzed water (pH 2.57, ORP 1161 mV, chlorine 66 mg/L); UST = ultrasound tank type (frequency = 40 kHz, power = 1.8 W/mL); USP: ultrasound probe type (frequency = 20 kHz, power = 46 W/mL); IR = gamma irradiation.

Table 3—Germination percentages[a] of broccoli seeds[b] treated with sterile water, acidic electrolyzed water, and ultrasound

	Treatment[c]									
	SW			AEW			UST		USP	
Time (min)	4 °C	23 °C	55 °C	4 °C	23 °C	55 °C	23 °C	55 °C	23 °C	55 °C
0.5									98.7a	97.4a
1							98.6a	98.3a	96.7a	95.9a
2	98.8a	98.6a	98.6a	98.2a	98.8a	97.9a	98.2a	97.4a	83.7b	85.2b
3									58.2c	75.1c
5	97.7ab	98.6a	98.0a	98.2a	98.8b	96.9a	98.4a	91.3b	23.7d	38.2d
10	97.4ab	98.6a	94.7a	97.9a	98.2a	93.7a	98.3a	39.0c	1.6e	7.4e
20	96.8b	98.9a	81.6b	97.8a	99.1a	75.4b	99.1a	4.2d		
30	97.8ab	98.4a	49.8c	97.6a	98.2a	41.2c	98.2a	0.8e		
60	96.7b	98.8a	7.8d	97.2a	98.8a	5.6d				

[a]Within treatment and temperature, values followed by different letters are significantly different ($P < 0.05$).
[b]Control = 99.3%.
[c]See Table 2.

of USP-treated samples. For alfalfa seeds, however, a higher germination for USP-treated seeds was recorded. The abnormality might be caused by the inability of USP treatment to bring the smaller but heavier alfalfa seeds into the reaction zone underneath the tip of the ultrasound probe. The AED in an ultrasound probe system is not uniform. The energy is localized within the small reaction zone at the tip of the probe (Mason and Lorimer 2002). Alfalfa and broccoli seeds had a density of less than unity; both seeds floated at the surface of the treatment chamber in the beginning of the treatment. During USP treatment, the seeds started to sink to the bottom. The emission of acoustic energy at the tip of the probe generates a current that can bring the seeds up into the liquid so that the seed kernels can be exposed to cavitation activity. The larger but lighter broccoli seeds may have less difficulties to be carried by the current into the liquid and in contact with the liquid jets so as to generate more damage and hence less germination.

Irradiation doses from 0.5 to 12 kGy were used to determine the effects of irradiation on germination percentage of alfalfa and broccoli seeds (Table 4). A decrease in germination by the IR treatment was not observed until the dose was greater than 4 and 8 kGy for alfalfa and broccoli seeds, respectively. The alfalfa seeds exhibited higher resistance to irradiation than the broccoli seeds, an observation consistent with that of Rajkowski and others (2003). Germination percentages of both seeds were severely affected by gamma irradiation at 12 kGy. Irradiation dose of up to 8 kGy has been approved by the U.S. Food and Drug Administration for inactivation of bacterial pathogens on seeds used for sprouting (US DHHS 2000). In the present study, we found that the germination percentages for alfalfa and broccoli seeds were significantly lowered when the doses were greater than 8 kGy. Also, a decrease in the length and thickness of radicals was observed at 8 kGy, an indication of the adverse effect of irradiation on the seeds. Earlier studies have shown yields of sprouts were reduced by irradiation at doses above 2 or 3 kGy (Rajkowski and others 2003; Fan and others 2004).

Population of *E. coli* O157:H7

The reduction in *E. coli* O157:H7 population in alfalfa seeds after treating with SW, AEW, UST, USP, and IR is presented in Table 5. Heat treatment alone at 55 °C reduced *E. coli* O157:H7 count by nearly 1.7 log CFU/g for the SW treatment. Log reduction in *E. coli* O157:H7 count was nearly doubled when alfalfa seeds were treated with AEW at 55 °C for 10 min compared to SW treatment at the same temperature and time, which is comparable to the 3.55 log reduction reported by Bari and others (2003b) for an AEW treatment in

Table 4—Germination percentages[a] of alfalfa and broccoli seeds treated with gamma irradiation

Dose (kGy)	Alfalfa seeds	Broccoli seeds
0	85.4a	99.3a
0.5	85.6a	98.5a
1	88.2a	98.1a
2	88.3a	98.9a
4	86.6a	98.5a
8	86.5a	92.5b
12	58.9b	19.8c

[a]Values followed by different letters are significantly different ($P < 0.05$).

combination with dry heat at 50 °C. In ultrasound treatments of alfalfa seeds, UST was less effective compared to USP at 23 °C but at 55 °C, the USP treatment only reduced *E. coli* O157:H7 population by 1.92 log CFU/g. Since the probe was placed 3 cm from the bottom of the treatment chamber, a strong vertical circulation of the liquid was observed during the USP treatment. The circulation entrained air into the liquid, especially at elevated temperature of 55 °C, which created additional interfaces in the liquid, impeded the propagation of ultrasound, and hence minimized cavitation activity in the treatment chamber. In addition, at 55 °C, an increase in vapor pressure would result in vapor-filled bubbles. The implosion of these bubbles was less powerful due to the cushioning effect of the vapor inside the bubbles (Manson and Lorimer 2002). *E. coli* O157:H7 was not recovered from alfalfa seeds after IR treatment at dose of 8 kGy, which was the only treatment that delivered an over 5-log reduction in this study. Thayer and others (2003) reported a 3.3-log inactivation of *E. coli* O157:H7 on alfalfa seeds by 2 kGy IR. Bari and others (2003a) also achieved over 5-log reduction in *E. coli* O157:H7 on alfalfa seeds after a dry heat treatment (50 °C for 1 h) and irradiation at doses was > 2 kGy.

E. coli O157:H7 cells recovered from broccoli seeds after treatments had similar but slightly lower population reductions compared to that of the alfalfa seeds (Table 6). AEW treatment at 55 °C lowered *E. coli* survival count by 3.27 log CFU/g, higher than the 2 ultrasonic treatments. IR again was the most effective among all treatments, with a reduction of *E. coli* O157:H7 population of 4.85 log CFU/g.

Table 5—The effect of sterile water, acidic electrolyzed water, ultrasound, and irradiation under the targeted germination percentage on reduction of *E. coli* O157:H7[a] population on alfalfa seeds

Treatments[b]		Population[c] (log CFU/g)	Reduction (log CFU/g)
No		5.03a	NA
SW	23 °C for 60 min	4.99a	0.04
	55 °C for 10 min	3.32b	1.71
AEW	23 °C for 60 min	4.43a	0.60
	55 °C for 10 min	1.67c	3.36
UST	23 °C for 30 min	4.68a	0.35
	55 °C for 5 min	2.09c	2.94
USP	23 °C for 10 min	3.48b	1.55
	55 °C for 5 min	3.11b	1.92
IR	8 kGy	0.00d	5.03

[a]The population of inocula suspension was 7.49 $\log_{10}$ CFU/mL.
[b]See Table 2.
[c]Values followed by different letters are significantly different ($P < 0.05$).

Table 6—The effect of sterile water, acidic electrolyzed water, ultrasound, and irradiation under the targeted germination percentage on reduction of *E. coli* O157:H7[a] population on broccoli seeds

Treatments[b]		Population[c] (log CFU/g)	Reduction (log CFU/g)
No treatment		5.21a	—
SW	23 °C for 60 min	5.01a	0.20
	55 °C for 10 min	3.67cd	1.54
AEW	23 °C for 60 min	4.25b	0.96
	55 °C for 10 min	1.94e	3.27
UST	23 °C for 30 min	5.08a	1.08
	55 °C for 5 min	3.26d	1.44
USP	23 °C for 2 min	4.13bc	1.13
	55 °C for 2 min	3.77bc	1.95
IR	8 kGy	0.36f	4.85

[a]The population of inoculum suspension was 7.56 $\log_{10}$ CFU/mL.
[b]See Table 2.
[c]Values followed by different letters are significantly different ($P < 0.05$).

Since the inactivation tests were conducted with treatment conditions for a minimal germination of 85%, the log reductions in Table 5 and 6 indicate the maximum microbial inactivation capacity of each treatment with acceptable seed viability. It can be seen that, with the 85% germination as a criterion, AEW and ultrasound alone, as well as the combination of AEW and ultrasound with heat at 55 °C, cannot provide an effective inactivation of *E. coli* O157:H7 cells inoculated on alfalfa and broccoli seeds. Multiple hurdles must be applied to achieve an over 5-log reduction or a complete elimination of *E. coli* cells.

Conclusions

With the treatment conditions selected for each hurdle to maintain a targeted germination percentage, which was 85% in this study, AEW and 2 ultrasound treatments (UST and USP) failed to produce an *E. coli* O157:H7 population reduction high enough to secure microbial safety. IR at dose of 8 kGy achieved a complete elimination of *E. coli* O157:H7 cells from alfalfa seeds but was accompanied by degradation in the yield of the sprouts. In order to eliminate *E. coli* O157:H7 from the 2 seeds used in this study, a combination of several hurdles must be used with treatment conditions determined again by germination tests.

Acknowledgments

This research was supported in part by the U.S. Dept. of Agriculture, Agricultural Research Service (USDA Specific Cooperative Agreement No. 58–1275–5-340) and the Illinois Agricultural Experiment Station. We thank Dr. Stoyan A Toshkov at Univ. of Illinois for his assistance in irradiation tests, Dr. Arun K Bhunia at Purdue Univ. for providing *E. coli* O157:H7 strains, and Crest Ultrasonics (Trenton, N.J., U.S.A.) for loaning the ultrasonic cleaning unit for use in this study.

References

Ariefdjohan MW, Nelson PE, Singh RK, Bhunia AK, Balasubramaniam VW, Singh N. 2004. Efficacy of high hydrostatic pressure treatment in reducing *Escherichia coli* and *Listeria monocytogenes* in alfalfa seeds. J Food Sci 69:117–20.
Bari ML, Nazuka E, Sabina Y, Todoriki S, Isshiki K. 2003a. Chemical and irradiation treatments for killing *Escherichia coli* O157:H7 on alfalfa, radish, and mung bean seeds. J Food Prot 66(5):767–74.
Bari ML, Sabina Y, Isobe S, Uemura T, Isshiki K. 2003b. Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on the surfaces of tomatoes. J Food Prot 66(4):542–8.
Beuchat LR, Scouten AJ. 2002. Combined effects of water activity, temperature, and chemical treatments on survival of *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J Appl Microbiol 92:382–95.
Breuer T, Benkel DH, Shapiro RL, Hall MM, Winnett WN, Linn MJ, Neimann J, Barrett TJ, Dietrich S, Downes FP, Toney DM, Pierson JL, Rolka H, Slutsker L, Griffin PM. 2001. A multistate outbreak of *Escherichia coli* O157:H7 infection linked to alfalfa sprouts grown from contaminated seeds. Emerg Infect Dis 7:77–82.
Fan X, Thayer DW, Sokorai KJB. 2004. Changes in growth and antioxidant status of alfalfa sprouts during sprouting as affected by gamma irradiation of seeds. J Food Protect 67:561–6.
Fett WF. 2006. Interventions to ensure the microbial safety of sprouts. In: Sapers GM, Gorny JR, Yousef AE, editors. Microbiology of fruits and vegetables. New York: Taylor & Francis. p 187–209.
Fett WF, Cooke PH. 2003. Reduction of *Escherichia coli* O157:H7 and *Salmonella* on laboratory-inoculated alfalfa seed with commercial citrus-related products. J Food Prot 66:1158–65.
Fett WF, Fu T-J, Tortorello ML. 2006. Seed sprouts: the state of microbiological safety. In: Matthews KR, editor. Microbiology of fresh produce. Washington, D.C.: ASM Press. p 167–219.
Kim C, Hung Y-C, Brackett RE, Lin C-S. 2003. Efficacy of electrolyzed oxidizing water in inactivating *Salmonella* on alfalfa seeds and sprouts. J Food Prot 66(2):208–14.
Lang MM, Ingham BH, Ingham SC. 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* O157:H7 from alfalfa seeds prior to sprouting. Int J Food Microbiol 58:73–82.
Leighton TG. 1994. The acoustic bubbles. London: Academic Press.
Manson TJ, Lorimer JP. 2002. Applied sonochemistry. Weinheim: Wiley-VCH Verlag GmbH.
[NACMCF] Natl. Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. Int J Food Microbiol 52:123–53.
Park CM, Hung YC, Doyle MP, Ezeike GOI, Kim C. 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. J Food Sci 66:1368–72.

Proctor ME, Hamacher M, Tortorello ML, Archer JR, Davis JP. 2001. Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. J Clin Microbiol 39:3461–5.
Rajkowski KT, Boyd G, Thayer DW. 2003. Irradiation D-values for *Escherichia coli* O157:H7 and *Salmonella* spp. on inoculated broccoli seeds and effects of irradiation on broccoli sprout keeping quality and seed viability. J Food Prot 66:760–6.
Scouten AJ, Beuchat LR. 2002. Combined effects of chemical, heat and ultrasound treatments to kill *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J Appl Microbiol 92:668–74.
Sharma RR, Demirci A. 2003. Treatment of *Escherichia coli* O157:H7 inoculated alfalfa seeds and sprouts with electrolyzed oxidizing water. Int J Food Microbiol 86: 231–7.
Sharma RR, Demirci A, Beuchat LR, Fett WF. 2003. Application of ozone for inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa sprouts. J Food Proc Preserv 27:51–64.
Soylemez G, Brashears MM, Smith DA, Cuppett SL. 2001. Microbial quality of alfalfa seeds and sprouts after a chlorine treatment and packaging modifications. J Food Sci 66(1):153–7.
Taormina PJ, Beuchat LR. 1999. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J Food Prot 62(4):318–24.
Thayer DW, Rajkowski KT, Boyd G, Cooke PH, Soroka DS. 2003. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* by gamma irradiation of alfalfa seed intended for production of food sprouts. J Food Prot 66:175–81.
[US DHHS] U.S. Dept. of Health and Human Services. 2000. Irradiation in the production, processing and handling of food. October 30, 2000. Docket 99F-2673 B final rule. US DHHS, Washington, D.C. Available from: http://vm.cfsan.fda.gov/~lrd/fcf179.html Accessed 2004 Oct 20.
Wade WN, Scouten AJ, McWatters KH, Wick RL, Demirci A, Fett WF. 2003. Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts and effects on sensory quality of sprouts. J Food Prot 66:44–51.
Wang H, Feng H, Luo Y. 2004. Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. Food Research Int 37:949–56.
Weissinger WR, Beuchat LR. 2000. Comparison of aqueous chemical treatments to eliminate *Salmonella* on alfalfa seeds. J Food Prot 63(11):1475–82.
Wuytack EY, Diels AMJ, Meersseman K, Michiels CW. 2003. Decontamination of seeds for seed sprout production by high hydrostatic pressure. J Food Prot 66:918–23.